Austin Chromatography

Open Access

Austin Publishing Group

Mini Review

# Current Status of the Techniques Used for Halitosis Analysis

**Yoneda M*, Suzuki N and Hirofuji T**
Department of General Dentistry, Fukuoka Dental College, Japan

***Corresponding author:** Yoneda M, Section of General Dentistry, Department of General Dentistry, Fukuoka Dental College, 2-15-1, Tamura, Sawara-ku, Fukuoka, 814-0193, Japan, Tel: +81-92-801-0411 (136); Fax: +81-92-801-4909; Email: yoneda@college.fdcnet.ac.jp

**Received:** December 06, 2014; **Accepted:** January 08, 2015; **Published:** January 09, 2015

**Abstract**

There are several types of halitosis, and accurate diagnosis of the type is important. Many gases are involved in oral malodor, and intraoral halitosis is most often caused by Volatile Sulfur Compounds (VSCs). To measure the concentrations of VSCs in mouth air, Gas Chromatography (GC) has been used, and this technique has contributed to much progress in halitosis research. However, GC instruments are large, expensive, and require a trained operator. To overcome these problems in daily use, portable sulfide monitors have been developed. Some of these monitors provide relatively accurate results, but they are no substitute for the accuracy of GC. Recently, two simple GCs were developed. These instruments are small and provide accuracy similar to traditional GC. This simpler instrumentation will ensure further progress in halitosis research.

**Keywords:** Gas chromatography; Halitosis; Volatile sulfur compound

## Introduction

Many people worry about breath odor, and often visit breath clinics for advice on this issue. Clinically, some patients who complain of halitosis have actual malodor, whereas others have almost no malodor. Diagnosis and treatment of halitosis involves simple classification into the following categories: genuine halitosis, pseudo halitosis, and halitophobia [1]. Genuine halitosis patients have oral malodor that is above a level perceivable to humans. Genuine halitosis can be further divided into physiologic halitosis and pathologic halitosis. The treatment of genuine halitosis primarily involves periodontal treatment, dental and oral care, and oral hygiene instructions. If the oral malodor is extra oral in origin, such as otolaryngological, respiratory, or digestive, the patient is referred to a medical doctor. On the other hand, patients with pseudo halitosis do not have bad breath. They worry about their breath odor, but by the examination and explanation by a doctor, they are free from their anxiety. Treatment of pseudo halitosis involves counseling that includes education and an explanation of the examination results to show that the intensity of the patient's malodor is not beyond socially acceptable levels. Halitophobia is a kind of mental problem. Halitophobia is characterized by a patient's persistent belief that he or she has halitosis despite reassurance, treatment, and counseling. Patients with halitophobia are referred to mental specialists.

Because there are several types of halitosis, diagnosis of the correct type is very important and accurate measurements of oral malodorous compounds are required. The simple and inevitable technique is oraganoleptic test. But this method lacks objectivity and reproducibility, and machine-based measurement was developed. The most common compounds associated with oral malodor of intraoral origin are volatile sulfur compounds (VSCs), such as hydrogen sulfide ($H_2S$), methyl mercaptan ($CH_3SH$), and dimethyl sulfide ($CH_3SCH_3$) [2]. Extra-oral halitosis is associated with compounds such as carbon monoxide, amines, ketones, and other volatile organic compounds in addition to VSCs [3]. Extra oral halitosis is important in medical field, but most of pathologic halitosis is associated with the VSCs in the oral cavity, so great efforts had been made to accurately measure the concentration of VSCs. In this article, we will summarize the methods for measuring breath odor - Gas Chromatography (GC), portable sulfide monitors, and simple GC.

### Organoleptic test

In halitosis, the perception of odor by other people is very important. To evaluate this, doctors carry out organoleptic techniques [4]. Organoleptic testing does not require any special devices, and is carried out by sniffing the patient's breath and scoring the level of oral malodor [1]. For reliable diagnosis, the oral malodor assessment should preferably be carried out on two or three different days. The recommended examination procedures are described below. Patients are instructed to abstain from the following: taking antibiotics for 3 weeks before the assessment, eating garlic, onions and spicy foods for 48 h before the assessment, and using scented cosmetics for 24 h before the assessment. Patients are further instructed to abstain from the following for 12 h before the assessment: ingesting any food or drink, their usual oral hygiene practices, using oral rinses and breath fresheners, and smoking. The oral malodor examiner, who should have a normal sense of smell, is required to refrain from drinking tea, coffee, or juice, and to refrain from smoking and using scented cosmetics before the assessment. Organoleptic testing is important and inevitable, but it is limited because the results have low objectivity and reproducibility. To overcome these limitations, a new method using a gastight syringe has been proposed [5]. Another problem with organoleptic testing is the difficulty in calibrating among examiners. Some halitosis studies have been performed without sufficient calibration, but organoleptic measurements can be calibrated among examiners with standards of VSC mixtures or individual VSCs [6].

### Gas chromatography (GC)

As an alternative to organoleptic testing, devices for measuring oral malodor have been developed. In the 1960s, chemical and Mass




**Citation:** Yoneda M, Suzuki N and Hirofuji T. Current Status of the Techniques Used for Halitosis Analysis. Austin Chromatogr. 2015;2(1): 1024.

Spectrometric (MS) analyses were applied to detect the odor in salvia headspace [7]. However, the sensitivity of the MS analyses was low and pre-trapping and concentration of volatiles from large volumes of mouth air was required to detect sulfur compounds. To overcome this problem, GC with flame ionization detection has been applied to measure oral malodor [8]. A number of volatile components have been identified by GC, but sulfur compounds, which are a major cause of oral malodor, have not been detected. Progress has been achieved with development of a Flame Photometric Detector (FPD) system [9]. This system has both high sensitivity and semi-specificity for sulfur compounds. Data obtained by Yaegaki's method [10] is considered the gold standard in measurements of oral malodor. In Yaegaki's method, samples of mouth air are analyzed using a conventional GC with a FPD equipped with a bandpass filter (at 393 nm). Because VSCs will be adsorbed to glass columns, Teflon columns packed with 25 % (1,2,3-tris (2-cyanoethoxy) propane (80–100 mesh) are used [11]. Yaegaki's method also requires precise injection of 10 mL of the sample gas to obtain accurate results. For this purpose, a six-port valve and 10 mL sample loop are included in the GC system [10].

## Portable sulfide monitors

It is generally accepted that a GC equipped with a FPD provides the most accurate oral malodor measurements among the available instrumentation. However, GCs are large, expensive, require trained operators, and have long run times. Consequently, many breath clinics use portable sulfide monitors. Many investigators and practicing dentists use an instrument called the Halimeter® (Interscan Corporation, Chatsworth, CA), to measure oral VSCs [12]. This instrument provides a digital readout of the total VSC concentration in gas aspirated from the oral cavity. While relatively inexpensive and easy to use, there is a lack of data concerning the accuracy of measurements obtained with this instrument. Furne et al. compared Halimeter® measurements with those obtained by a GC equipped with a sulfur detector [13]. In this case, the Halimeter® measurements of peak and plateau concentrations of oral VSCs did not perfectly mirror the results of GC analysis. The appreciable discrepancy between the Halimeter® and GC results indicates that when precise knowledge of VSC concentrations is required, GC analysis is the best technique for this. However, the sulfide detector responds linearly to $H_2S$ and $CH_3SH$, which are the primary VSCs in breath gas. Thus, while lacking perfect accuracy, the sulfide detector provides useful data for clinical studies of oral malodor.

Another sulfide monitor (Breathtron®, New Cosmos Electric, and Osaka, Japan) is also used among dental practitioners. Sopapornamorn et al. examined the association between oral malodor and measurements obtained using the Breathtron® [14]. The log values of VSC concentrations measured by the Breathtron® were significantly correlated with organoleptic ratings, and also with log values of $H_2S$, $CH_3SH$, and $(CH_3)_2S$ concentrations determined by GC ($P<0.01$). Using the results of organoleptic tests and GC to classify subjects into normal and malodor groups, the Breathtron®'s sensitivity was shown to be more than 79 % and the specificity was 61–73 %. Another group compared the Breathtron® readings and GC measurements, and found a significant correlation ($R=0.89$, $y=2.13x$, $P<0.0001$) [10]. However, they also experienced frequent misdiagnosis, and the detectors could sometimes not read VSC concentrations at threshold levels accurately.

Recently, another sulfide monitor (BB Checker) has become available, but the overall sensitivity and specificity of this device do not exceed 50 % [15]. Therefore, among the portable monitors, the Halimeter® and Breathtron® are the most appropriate devices for general dental practitioners because they are easy to handle and provide similar results to GC.

## Simple GC

Portable sulfide monitors are widely used for quantitative measurements of oral malodor. These monitors have sufficient sensitivity to detect $H_2S$, but also detect other volatiles in the breath, even ones that are not malodorous [13,16]. Recently, two simple gas chromatographs were developed. One of these is the OralChroma™ (Abilit, Osaka, Japan), which consists of a Teflon column packed with 25 % oxydipropionitrile supported on Celite (GL Science, Tokyo, Japan) [17]. VSCs are detected by a newly invented indium oxide ($In_2O_3$) Semiconductor Gas Sensor (SGS). It only needs electricity and the apparatus can are used everywhere. It does not need carrier gas such as helium or nitrogen, like in standard gas chromatography. It uses the room air as a carrier gas for the chromatographic column. Murata et al. compared GC-SGS measurements with those obtained using GC-FPD, and the GC-SGS results agreed with the GC-FPD results [18]. Pearson correlation coefficients between concentrations measured with both methods yielded high values, including $R=0.821$ for $H_2S$ ($P<0.0001$), $R=0.870$ for $CH_3SH$ ($P<0.0001$), and $R=0.770$ for $(CH_3)_2S$ ($P<0.0001$). These values indicate sufficient performance as a measuring device for VSCs. While ethanol, component of most mouthwashes, affects accurate measurements with the GC-SGS, this effect is much lower than in a sulfide monitor. Based on the hardware, the OralChroma™ could become the apparatus of choice in the field of halitosis. However, there are some issues with the OralChroma™ software, such as incorrect assignments of the positions of VSCs in the chromatogram. Tangerman et al. applied a modified protocol [19] to improve the sensitivity of the OralChroma™ in the measurement of VSCs. The OralChroma™ is currently applied in research and clinical diagnosis [20].

Recently, another portable GC called the Twin Breasor™ (GC Co., Tokyo, Japan) was developed. Yaegaki et al. [10] compared the Twin Breasor™ with traditional GC, and reported that almost complete correlations were found for $H_2S$ ($R=0.97$, $y=107x–0.04$, $P<0.0001$), and for $CH_3SH$ ($R=0.90$, $y=0.95x$, $P<0.0001$). For $H_2S$ and $CH_3SH$ measurements, the coefficients of determination were 0.94 and 0.81, respectively. Although the Twin Breasor™ cannot measure concentration of $(CH_3)_2S$, which is a minor component of mouth air, measurements for the two major VSCs ($H_2S$ and $CH_3SH$) were very accurate. In addition, while the OralChroma™ requires a personal computer, the Twin Breasor™ can work without a personal computer. Currently, a second generation Twin Bressor II™ (iSenLab, Seoul, Korea) is available, and this machine cuts the measurement time of the first generation machine at least in half.

As discussed above, the OralChroma™ and Twin Breasor™ (Twin Bressor II™) are simple GCs that can measure the concentrations of VSCs. The results are similar to those obtained by traditional GC. Therefore, these simple GCs could be used as reliable VSC measuring devices.

**Table 1:** Comparison of the techniques used for halitosis analysis.

| | | Organoleptic test | GC[a] with FPD[b] | Portable sulfide monitor | Simple GC |
|---|---|---|---|---|---|
| Advantage | | Close to human sensateion | 3 VSCs[c] can be measured | Easy to handle | No carrier gas required |
| | | No machine requiered | Accurate enough for gold standard | Relatively reasonable | Accurate |
| Disadvantage | | Variable among examiners | Expensive | Sensitive to alchohol | More expensive than portable sulfide monitors |
| | | Low reproducibility | Require carrier gas and handling technique | Cannot measure each VSC concentraion | Cannot or difficult to measure $(CH_3)_2S$ |

[a] gas chromatograpy
[b] flame photometric detector
[c] volatile sulfur compounds

## Conclusion

There are several types of halitosis, and accurate diagnosis of the type is very important [21]. Oral malodor can have a number of causes, and intraoral malodor is most often caused by VSCs. Therefore, accurate measurement of VSCs concentrations in mouth air is required in halitosis diagnosis, and there are several methods (Table 1). Organoleptic testing is inevitable but its objectivity and reproducibility is not high. To measure VSCs concentrations accurately, GC instruments have been introduced in halitosis clinics and in research, and much progress has been made with this technology in this area. However, GC instrumentation is large, expensive, and requires technical knowledge to operate. To overcome these issues, many portable sulfide monitors have been developed. Some of these monitors provide relatively accurate results and they can be used to diagnose halitosis. Unfortunately, sulfide monitors are sensitive to alcohol of mouthwash, and they cannot distinguish among the different VSCs. Recently, two simple GCs were produced, and these instruments have similar accuracy to traditional GC with FPD. These GCs are not large, and they are cheaper than the traditional GCs. Moreover, sample handling is easier and trained operators are not required. While traditional GC greatly facilitated advances in halitosis research and the data obtained by GC-FPD is still the gold standard, these new simple GCs will also prove useful in clinics and laboratories and contribute to further developments in this area.

## References


1. Yaegaki K, Coil JM. Examination, classification, and treatment of halitosis; clinical perspectives. J Can Dent Assoc. 2000; 66: 257-261.
2. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontol. 1977; 48: 13-20.
3. Tangerman A, Winkel EG. Extra-oral halitosis: an overview. J Breath Res. 2010; 4: 017003.
4. Yaegaki K, Coil JM. Diagnosis of halitosis by utilizing questionnaire and organoleptic measurement. Quintessence. 1999; 18: 745-753.
5. Kim DJ, Lee JY, Kho HS, Chung JW, Park HK, Kim YK. A new organoleptic testing method for evaluating halitosis. J Periodontol. 2009; 80: 93-97.
6. Nachnani S, Majerus G, Lenton P, Hodges J, Magallanes E. Effects of training on odor judges scoring intensity. Oral Dis. 2005; 11 Suppl 1: 40-44.
7. Tonzetich J, Richter VJ. Evaluation of Volatile Odoriferous Components of Saliva. Arch Oral Biol. 1964; 9: 39-46.
8. Larsson Bt. Gas Chromatography of Organic Volatiles in Human Breath and Saliva. Acta Chem Scand. 1965; 19: 159-164.
9. Brody SS, Chaney JE. Flame photometric detector. The application of a specific detector for phosphorous and for sulfur compounds – sensitive to subanogram quantities. J Gas Chromatogr. 1966; 4: 42-46.
10. Yaegaki K, Brunette DM, Tangerman A, Choe YS, Winkel EG, Ito S, et al. Standardization of clinical protocols in oral malodor research. J Breath Res. 2012; 6: 017101.
11. Tonzetich J. Direct gas chromatographic analysis of sulphur compounds in mouth air in man. Arch Oral Biol. 1971; 16: 587-597.
12. Rosenberg M, McCulloch CA. Measurement of oral malodor: current methods and future prospects. J Periodontol. 1992; 63: 776-782.
13. Furne J, Majerus G, Lenton P, Springfield J, Levitt DG, Levitt MD. Comparison of volatile sulfur compound concentrations measured with a sulfide detector vs. gas chromatography. J Dent Res. 2002; 81: 140-143.
14. Sopapornamorn P, Ueno M, Vachirarojpisan T, Shinada K, Kawaguchi Y. Association between oral malodor and measurements obtained using a new sulfide monitor. J Dent. 2006; 34: 770-774.
15. Dadamio J, Laleman I, De Geest S, Vancauwenberghe F, Dekeyser C, Coucke W, et al. Usefulness of a new malodour-compound detection portable device in oral malodour diagnosis. J Breath Res. 2013; 7: 046005.
16. Van Steenberghe D. Breath malodor. Curr Opin Periodontol. 1997; 4: 137-143.
17. Hamada M, Koda H, Onaga K, Tanaka K, Okabayashi T, Itoh T, et al. Portable oral malodor analyzer using highly sensitive $In_2O_3$ gas sensor combined with a simple gas chromatography system. Anal. Chim Acta. 2003; 475: 27-35.
18. Murata T, Rahardjo A, Fujiyama Y, Yamaga T, Hanada M, Yaegaki K, et al. Development of a compact and simple gas chromatography for oral malodor measurement. J Periodontol. 2006; 77: 1142-1147.
19. Tangerman A, Winkel EG. The portable gas chromatograph OralChroma™: a method of choice to detect oral and extra-oral halitosis. J Breath Res. 2008; 2: 017010.
20. Awano S, Takata Y, Soh I, Yoshida A, Hamasaki T, Sonoki K, Ohsumi T. Correlations between health status and OralChroma™-determined volatile sulfide levels in mouth air of the elderly. J Breath Res. 2011; 5: 046007.
21. Suzuki N, Yoneda M, Hirofuji T. Evidence-based control of oral malodor. Mandeep Virdi, editor. In: Oral Health: In Tech. [In Press].


Austin Chromatogr - Volume 1 Issue 5 - 2014
ISSN 2379-7975 | www.austinpublishinggroup.com


**Citation:** Yoneda M, Suzuki N and Hirofuji T. Current Status of the Techniques Used for Halitosis Analysis. Austin Chromatogr. 2014;1(5): 1024.